REMARKS

In the Final Action dated September 16, 2008, claims 74-113 are pending, of which claims 74-89 are withdrawn from consideration as directed to non-elected subject matter. The Examiner has withdrawn the requirement for species election set forth in the Office Action dated May 15, 2008. Claims 90-113 are examined on the merits and are rejected.

Specifically, claims 107-113 are rejected under 35 U.S.C. §112, second paragraph, as indefinite. Claims 90-96 and 104-113 are rejected under 35 U.S.C. §102(e) as anticipated by Gerngross (U.S. Patent Publication No. 2002/0137134), as evidenced by JP 8-336387. Claims 90-91, 93-96, 104-108, 110 and 112-113 are rejected under 35 U.S.C. §103(a) as unpatentable over Martinet et al. (*Biotechnology Letters* 20: 1171-1177, 1998) in view of JP 8-336387, as evidenced by Choi et al. (*PNAS* 100: 5022-5027, 2003). Claims 97-106 are rejected under 35 U.S.C. §103(a) as unpatentable over Martinet et al. in view of JP 8336387, as evidenced by Choi et al., as applied to claims 90-91, 93-96, 104-108, 110 and 112-113 above, and further in view of Trombetta et al. (*J. Biol. Chem.* 271: 27509-27516, 1996) and Chiba et al. (*J. Biol. Chem.* 273: 26295-26304, 1998) Claims 90-96 and 105 are rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claim 9 of U.S. Patent No. 7, 252,933. Claims 90-96 and 105 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 39, 41-59 of copending Application No. 10/713,970. Claims 90-113 are rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225.

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim Amendments

Independent claims 90 and 107 have been amended to define that the α-1,2-mannosidase or the functional part thereof is genetically engineered to contain an ER-retention signal, which is a feature previously delineated in dependent claims 91 and 108, respectively. Claims 91 and 108 are therefore canceled.

Claims 90 and 107 have also been amended to define that the strain produces $Man_5GlcNAc_2$ as a predominant N-glycan structure or a predominant intermediate N-glycan structure. Support for "$Man_5GlcNAc_2$ as a predominant N-glycan structure" is found in the specification, especially on page 10, lines 21-24 (referring to $Man_5GlcNAc_2$ as the "major glycan") and Panel 4 of Figure 10. Support for $Man_5GlcNAc_2$ as an "intermediate N-glycan" is implicit in the specification, and is also explicitly found on, e.g., page 14, lines 17-19 of the specification (referring to $Man_5GlcNAc_2$ as an "intermediate" for hybrid- and complex-type sugar chains). Claims 96 and 113 are canceled in view of the amendments to claims 90 and 107.

Further, claim 107 has been amended to address certain alleged indefiniteness.

Moreover, new claims 114-115 are added to define that the Och1 disruption is the sole genetic disruption of the Golgi mannosyl transferases acting in N-glycosylation of the strain. Although the specification discloses that the strain can be further modified to disrupt additional Golgi mannosyl transferases (e.g., page 13, lines 21-24, and page 27, lines 8-9), disruption of Och1 alone evidenced desirable results (page 45 and Figure 10) and certainly represents a preferred embodiment of the invention.

It is respectfully submitted that no new matter is introduced by the forgoing amendments.

35 U.S.C. §112, Second Paragraph.

Claims 107-113 are rejected under 35 U.S.C. §112, second paragraph, as indefinite.

According to the Examiner, claims 107-113 are incomplete for omitting an essential element. That is, the *Pichia* strain must also be transformed with a nucleotide sequence coding for a heterologous glycoprotein so that the transformed cells of said *Pichia* strain can produce the heterologous glycoprotein. Additionally, the Examiner states that independent claim107 recites the limitation "said functional part thereof" in lines 3 and 5 of the claim, which lacks antecedent basis.

In response, independent claim 107 has been amended to add the clause, "wherein said cells are also transformed with a nucleotide sequence coding for said heterologous glycoprotein", and to replace the first recitation of "said functional part thereof" with "a functional part thereof". In view the amendments to claim 107, the rejection under 35 U.S.C. §112, second paragraph, is overcome. Withdrawal of the rejection is respectfully requested.

35 U.S.C. §102(e)

Claims 90-96 and 104-113 are rejected under 35 U.S.C. §102(e) as anticipated by Gerngross (U.S. Patent Publication No. 2002/0137134), as evidenced by JP 8-336387.

In response to Applicants' previous submission, the Examiner argues that Gerngross does not teach explicitly that *T. reesei* α-1,2-mannosidase should not be used. The Examiner considers the reference to teach *T. reesei* α-1,2-mannosidase as capable of producing $Man_5GlcNAc_2$ *in vivo* even though it is not efficient. The Examiner also notes that the rejected claims do not require the genetically engineered *Pichia* yeast strain to produce $Man_5GlcNAc_2$.

In the first instance, Applicants maintains that the teachings of Gerngross are inadequate to clearly anticipate the claimed strain that requires a *Pichia* strain to express *T. reesei* α-1,2-mannosidase or a functional part thereof and to include an Och1 genomic disruption. One would have to pick and select pieces of prophetic teachings compiled in separate contexts in Gerngross to arrive at the claimed strain. Even more, one would have to pick and select against the explicit teaching by Gerngross that the α-1,2 mannosidase should have an optimal pH that is between 5.1 and 8.0 (which would exclude the *T. reesei* enzyme for use in a *Pichia* strain) in order to obtain $Man_5GlcNAc_2$ in high yield. Further, Gerngross lists several enzymes involved in hypermannosylation, including Och1, Mnn4, Mnn6, and Mnn1, and does not disclose that a disruption of Och1 alone in a *Pichia* strain would be sufficient to obtain a significant amount of Man_8 and ultimately Man_5 N-glycans.

In order to clearly delineate preferred features of the present invention, Applicants have amended independent claims 90 and 107 such that the genetically engineered *Pichia* yeast strain produces $Man_5GlcNAc_2$ as a predominant glycoform or intermediate glycoform. Gerngross does not provide adequate teaching for a *Pichia* strain that produces $Man_5GlcNAc_2$ as a predominant glycoform or glycoform precursor by expressing *T. reesei* α-1,2-mannosidase or a functional part thereof and disrupting the Och1 gene. Additionally, Applicants have also presented new dependent claims 114-115, which define the Och1 disruption as the sole genetic modification of the Golgi mannosyltransferases acting in N-glycosylation of the strain. Neither Gerngross nor the post-filing reference, Choi et al., discloses that an Och1 disruption alone would be sufficient to obtain any significant amount of Man_8 and ultimately Man_5 N-glycan as a predominant glycoform or glycoform precursor.

Moreover, Applicants respectfully submit that Gerngross is not entitled to a priority date to be an effective prior art under §102(e) relative to the claimed invention.

Specifically, the cited Gerngross reference, US 2002/0137134, was filed on June 27, 2001, and claims priority from Provisional Application 60/214,358, filed on June 28, 2000, Provisional Application 60/215,638, filed on June 30, 2000, and Provisional Application 60/279,997, filed on March 30, 2001. Applicants provide herewith a copy of Gerngross' first Provisional Application 60/214,358 (**Exhibit 1**). This provisional application consists of only 5 pages. The only text of any relevance to the claimed invention appears on page 3, lines 5-15, which is reproduced herein below:

> "Genes may be transformed into the host cells expressing the recombinant glycoproteins which encode any combination of the following: sialyltransferases, mannosidases, fucosyltransferases, galactosyltransferases, glucosyltransferases, GlcNAc transferases, ER and Goli specific transporters ..... Genes may be deleted which encode enzymes such as enzymes homologous to OCH1, MNN1 (putative mannosyltransferases in *S. cerevisiae*) and other enzymes known to be characteristic of non-human glycosylation reactions."

There is no mention of α-1,2-mannosidase, much less *T. reesei* α-1,2-mannosidase, or of a combination of expression of α-1,2-mannosidase in conjunction with an OCH1 disruption to produce Man_5 N-glycans. Therefore, Applicants respectfully submit that this first provisional application of Gerngross is wholly deficient with respect to the teachings relied upon by the Examiner in rejecting the instant claims. Gerngross is simply ***not*** entitled to the priority date of June 28, 2000. In contrast, the priority document of the present application, Provisional Application No. 60/215,676, filed June 30, 2000, explicitly discloses a methylotrophic yeast strain such as a *Pichia* strain which expresses *T. reesei* α-1,2-mannosidase and contains an Och1 disruption. See, e.g., claim 21, page 9, 3rd paragraph from the bottom, and page 21, 3rd paragraph

from the top, of Provisional Application No. 60/215,676. Therefore, even assuming, *pro arguendo*, that Gerngross is entitled to the priority of its second provisional application (filed on June 30, 2000), this second provisional application of Gerngross is not "prior" in filing relative to the priority date of the present application. Therefore, Gerngross does not qualify as prior art under §102(e) relative to the claimed invention.

In view of the foregoing, the §102(e) rejection based on Gerngross is overcome and withdrawal thereof is respectfully requested.

35 U.S.C. §103

Claims 90-91, 93-96, 104-108, 110 and 112-113 are rejected under 35 U.S.C. §103(a) as unpatentable over Martinet et al. (*Biotechnology Letters* 20: 1171-1177, 1998) in view of JP 8-336387, as evidenced by Choi et al. (*PNAS* 100: 5022-5027, 2003).

Claims 97-106 are rejected under 35 U.S.C. §103(a) as unpatentable over Martinet et al. in view of JP 8336387, as evidenced by Choi et al., as applied to claims 90-91, 93-96, 104-108, 110 and 112-113 above, and further in view of Trombetta et al. (*J. Biol. Chem.* 271: 27509-27516, 1996) and Chiba et al. (*J. Biol. Chem.* 273: 26295-26304, 1998).

The Examiner has acknowledged Applicants' argument that the discussion of an och1 mutant in Martinet et al. was in reference to a *S. cerevisiae* strain. The Examiner states that although the glycosylation pathways of *S. cerevisiae* and *P. pastoris* are significantly different, inactivated mutations including deletion of endogenous Och1 in either *S. cerevisiae* or *P. pastoris* strain would prevent or reduce hyperglycosylation. Further, with respect to the post-filing reference of Choi et al. (2003), which was provided by Applicants to show that Man_9 is still a predominant N-glycan form in a Och1 mutant of *Pichia*, the Examiner states that Choi et

al. only showed the released N-linked glycans analysis from an Ochl-deleted *P. pastoris* strain, not from an Och1-deleted *P. pastoris* strain also expressing an exogenous α-1,2-mannosidase. Furthermore, the Examiner points out that in Choi et al., it is stated that fungal α-1,2-mannosidases with acidic pH optima, when expressed as fusions with the leader library, generally resulted in low $Man_5GlcNAc_2$ yields. In addition, the Examiner states that the rejected claims do not require the genetically engineered *Pichia* yeast strain to produce $Man_5GlcNAc_2$ to any degree of efficiency or any particular fraction of produced glycoproteins having the $Man_5GlcNAc_2$ structure.

Responsive to the Examiner's position, Applicants first respectfully submit that independent claims 90 and 107 have been amended such that the *T. reesei* α-1,2-mannosidase or a functional part thereof contains an ER-retention signal, and the genetically engineered *Pichia* yeast strain produces $Man_5GlcNAc_2$ as a predominant glycoform or intermediate glycoform. Applicants respectfully submit that the combination of Martinet et al. and JP 8336387, which is the premise of both §103(a) rejections above, does not render the presently claimed invention obvious. Specifically and as further elaborated hereinbelow, the cited references do not provide adequate teaching or suggestion for a *Pichia* strain that expresses *T. reesei* α-1,2-mannosidase or a functional part thereof localized intracellularly to the ER and that contains an Och1 genomic disruption, let alone a *Pichia* strain having these genetic modifications that produces $Man_5GlcNAc_2$ as a predominant glycoform or a predominant intermediate glycoform. Further, as evidenced by the cited references and other contemporaneous art references, as discussed in details below, there was substantial uncertainty at the relevant time such that those skilled in the art would not have had a reasonable expectation of success even if they had motivation to attempt to arrive at the presently claimed invention.

With respect to Martinet et al., it is important to recognize that the reference discloses making two forms of *T. reesei* α-1,2-mannosidase: a secreted form and an intracellular form. The secreted form of the enzyme was detected primarily in the medium. Given the expression of this secreted form, the glycan on neuraminidase (NA) was shown to be trimmed from $Man_{14}GlcNAc_2$ to smaller form (see last paragraph of page 174 to the first few lines of page175). When the *T. reesei* α-1,2-mannosidase was expressed as a fusion "chimeric" protein with the ER-localization signal of MNS1 segment and was therefore targeted to the ER, the extent of glycosylation was not only worse than those obtained with the secreted form, but worse than a wild type host with no *T. reesei* α-1,2-mannosidase at all -- more higher mannose forms were observed with the chimeric enzyme. For example, it is stated in the reference:

> "Expression of chimeric α-1,2-mannosidase had a rather opposite effect on the NA glycosylation content, since small glycans (<$Man_{14}GlcNAc_2$), present on wild type NA, could no longer be detected after treatment (Fig. 2B). Similar results were obtained with HA...." Page 1175, middle of right column (emphasis added).

> "Co-expression of heterologous *T. reesei* α-1,2-mannosidase, secreted in the culture medium of *P. pastoris*, maybe compared to *in vitro* α-1,2-mannosidase digestion, resulting in more extensively processed oligosaccharides. On the contrary, chimeric MNS1 α-1,2-mannosidase, which is retained intracelluarly, rather stimulates mannosyltransferase activity by removal of terminal α-1,2-mannose residues." Page 1175, last 3 lines to page 1176, first 5 lines (emphasis added).

Applicants respectfully submit that a prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore and Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983). Viewing the Martinet et al. reference in its entirety, those skilled in the art would not have been motivated to select the portion of the reference relating to the intracellular expression of the α-1,2-mannosidase, and to further modify this teaching by combining with an Och1 disruption as

taught by JP 8-336387. Martinet et al. clearly teach that an intracellularly expressed α-1,2-mannosidase was not effective, and suggest that one is likely to trim the glycan more effectively with an extended exposure to the α-1,2-mannosidase enzyme following co-secretion from the host.

Further, even assuming, *pro arguendo*, that those skilled in the art were to combine intracellular expression of the α-1,2-mannosidase enzyme with an Och1 disruption, those skilled in the art would still not have had a reasonable expectation of success in significantly reducing hyperglycosylation, much less in producing $Man_5GlcNAc_2$ as a predominant glycoform or a predominant intermediate glycoform.

First, with respect to JP 8-336387, this reference discloses a mutant *Pichia* strain having its Och1 gene disrupted. The only evaluation of the effect of the Och1 disruption on glycan size was through an SDS-PAGE analysis of a single glycoprotein expressed in the mutant strain as compared to the wild type strain, showing a lower apparent molecular weight of the protein in the mutant. However, this single protein appeared as a smear over at least a range of 15 kDa on the gel, indicating significant heterogeneity in its glycosylation. Therefore, there is no showing in this reference of production of Man_8 glycoforms, much less Man_8 as a predominant glycoform, which could be converted to Man_5 by an α-1,2-mannosidase. Therefore, JP 8-336387 would not have provided those skilled in the art with any reasonable expectation of success in obtaining a meaningful amount of $Man_5GlcNAc_2$ based on an Och1 disruption combined with expression of an α-1,2-mannosidase.

Moreover, Applicants submit that the art evidenced significant unpredictability in the field at the relevant time. For example, several Golgi mannosyltransferases (including α-1,6-mannosyltransferase encoded by Och1) were reported for *S. cerevisiae*. Nakanishi-Shindo et al.

(*J. Boil. Chem.* 268: 26338-26345, 1993, attached hereto as **Exhibit 2**) reported that a double mutant of *och1 mnn1* (with disruptions in both OCH1 and MNN1) was required to obtain Man8 as a predominant glycoform in *S. cerevisiae*, whereas a *och1* single mutant produced a mixture of Man_8, Man_9 and Man_{10} N-glycans, with Man_9 being the dominant form. Chiba et al. (*J. Biol. Chem.* 273: 26298-26304, 1998, attached as **Exhibit 3**) reported obtaining $Man_5GlcNAc_2$ in *S. cerevisiae* using a *och1 mnn1 mnn4* triple mutant and an α-1,2-mannosidase. The enzyme encoded by Mnn4 results in mannosylphosphorylation of the baker yeast's N-glycans, a modification which was known to be present in about 30% of N-glycans in *Pichia* (Grinna et al., Yeast. 1989 Mar-Apr;5(2):107-15, attached as **Exhibit 4**; e.g., the abstract). Therefore, considering the findings with *S. cerevisae*, those skilled in the art would not have reasonably expected that a disruption of the Och1 gene, *alone*, would have led to a production of $Man_8GlcNAc_2$ in an effective amount to permit the subsequent production of $Man_5GlcNAc_2$ as a predominant glycoform. In this connection, the Examiner's attention is directed to new claims 114-115, which define the Och1 disruption to be the sole genetic modification of the Golgi mannosyltransferases acting in N-glycosylation of the strain.

Further, inactivation of the Och1 gene in *S. cerevisiae* was also severely detrimental to cell viability. See, e.g., Nagasu et al. (Yeast 8: 535-547, 1992, attached hereto as **Exhibit 5**) (see the abstract of the article, for example). As commented by Vervecken et al. (*Applied Environmental. Microbiology* 70: 2639-2646, 2004, attached as **Exhibit 6**), "N-glycan homogenization in baker yeast has been an inefficient process, and the strains obtained might be too weak for general use in glycan engineering." (Page 2640, left column, second paragraph).

In view of the foregoing, Applicants respectfully submit that the subject matter as presently claimed is not obvious in view of Martinet et al. and JP 8-336387. Withdrawal of the rejections under 35 U.S.C. §103(a) is respectfully requested.

Nonstatutory Obviousness-Type Double Patenting

Claims 90-96 and 105 are rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claim 9 of U.S. Patent No. 7, 252,933. Applicants note that U.S. Patent No. 7, 252,933 has issued from Application Serial No. 10/185,475, filed after the present application.

Claims 90-96 and 105 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 39 and 41-59 of copending Application No. 10/713,970. Applicants note that the rejection is provisional as the conflicting application has not issued.

Claims 90-113 are rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225. Applicants note that U.S. Patent No. 6,803,225 has issued from the parent of the present application.

Applicants intend to address the above non-statutory double patenting rejections once the claims are found otherwise allowable, and will file appropriate terminal disclaimers in due course if necessary.

Conclusion

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

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